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Review article

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Cell senescence in liver diseases: pathological mechanism and theranostic opportunity

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Abstract

The liver is not oblivious to the passage of time, as ageing is a major risk factor for the development of acute and chronic liver diseases. Ageing produces alterations in all hepatic cells, affecting their phenotype and function and worsening the prognosis of liver disease. The ageing process also implies the accumulation of a cellular state characterized by a persistent proliferation arrest and a specific secretory phenotype named cellular senescence. Indeed, senescent cells have key roles in many physiological processes; however, their accumulation owing to ageing or pathological conditions contributes to the damage occurring in chronic diseases. The aim of this Review is to provide an updated description of the pathophysiological events in which hepatic senescent cells are involved and their role in liver disease progression. Finally, we discuss novel geroscience therapies that could be applied to prevent or improve liver diseases and age-mediated hepatic deregulations.

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Key points

- Senescent hepatocytes undergo morphological changes and metabolic alterations, negatively affecting liver function and promoting disease progression.
- Structural and functional changes in senescent liver sinusoidal endothelial cells contribute to liver disease.
- In the initial stages of liver disease, hepatic stellate cells undergo senescence, facilitating liver regeneration and fibrosis regression. Conversely, during cirrhosis, the increase in their number induces inflammation, promoting hepatocellular carcinoma (HCC).
- Senescence has a role in the development and progression of metabolic dysfunction-associated steatotic liver disease and viral hepatitis, contributing to liver injury, inflammation, fibrosis and HCC.
- Senescence can protect against HCC, inhibiting the growth of damaged cells; it can also lead to HCC progression by promoting inflammation and creating a pro-tumoural microenvironment within the liver.
- Senotherapeutic strategies, drug repurposing, and lifestyle interventions hold potential as therapies for liver disease by targeting and eliminating senescent cells and counteracting the harmful effects of senescence and ageing.

Introduction

Cellular stress can present numerous challenges to cells, disrupting cellular homeostasis. Indeed, and depending on the severity of the damage, the response to these various stress insults can range from complete restoration of structural and functional integrity to the activation of cell death mechanisms. Certainly, within the spectrum delineated by these two contrasting conditions, actively dividing cells can transition into alternative states, including cellular senescence[1].

Senescence is characterized as a cellular state involving cell cycle arrest and macromolecular damage, which coexists with a viable and functional phenotype. This state is further distinguished by an active secretory phenotype and a deregulated metabolic profile[1]. Cellular senescence, which emerges as a pivotal molecular mechanism aimed at inhibiting cell replication in response to cellular damage or following a finite number of cell division cycles, might also contribute to the development of liver diseases[2,3]. Within the context of liver cells, hepatocytes, liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs) and Kupffer cells are all subjected to different changes in their phenotype due to senescence, caused by either liver damage or age[4]. Indeed, ageing brings about notable changes in the liver that, collectively, contribute to a diminished capacity to maintain homeostasis, rendering it more vulnerable to injury and leading to a poor prognosis for liver diseases, such as metabolic dysfunction-associated steatohepatitis (MASH, formerly known as non-alcoholic steatohepatitis[5]), alcohol-related liver disease (ALD) and viral infections. Furthermore, the regenerative abilities of the liver are adversely affected by ageing, resulting in a reduced capacity for proliferation, which subsequently affects the survival rate following liver transplantation in older individuals[6,7] (Box 1).

For these reasons, it is crucial to understand the intricate mechanisms underlying liver senescence and unravel the precise triggers and pathways leading to this distinctive cellular phenotype. Hence, identifying novel therapeutic targets and developing innovative interventions to mitigate the detrimental consequences of liver senescence are essential.

This Review provides a comprehensive overview of cell senescence in liver diseases. It explores the changes occurring in hepatocytes and the main liver non-parenchymal cells during senescence, the importance of senescence in liver pathobiology, and the potential therapeutic strategies targeting cell senescence as novel treatments for liver diseases. Although senescence is also relevant in other liver cell types, such as cholangiocytes, this Review focuses on the four main cell types within the hepatic sinusoid.

The basics of liver structures and their role in liver diseases

The liver is a complex and highly specialized organ with many important functions, including filtering toxins from the blood, producing bile to aid in the digestion of fats, and storing and regulating nutrients, hormones and other substances. It consists of small units called lobes composed of parenchymal and non-parenchymal liver cells. A fibrous capsule surrounds the liver and receives its blood supply from the hepatic artery and the portal vein[8].

Acute or chronic deregulation of liver cells in response to exogenous stress factors can lead to liver disease, which can further develop into hepatocellular carcinoma (HCC)[9]. Indeed, liver disease can develop through a variety of mechanisms, such as: (1) chronic injury from exposure to toxins, viruses or other substances can lead to the gradual accumulation of damage and the development of liver disease over time; (2) chronic inflammation can lead to scarring and fibrosis, which can impair liver function and increase the risk of liver disease; (3) excessive fat accumulation in the liver might lead to liver damage and the development of metabolic dysfunction-associated steatotic liver disease (MASLD, formerly known as non-alcoholic fatty liver disease[5]); (4) genetic factors caused by genetic mutations that lead to problems with liver function or the development of liver disease; (5) immune response, leading to inflammation and damage to the liver; and (6) congenital defects, such as biliary atresia[10]. Thus, the development of liver disease can be induced by a combination of genetic and environmental factors, including lifestyle factors such as diet, alcohol consumption and exposure to drugs.

Liver disease is a major global health issue, affecting millions of people worldwide. Liver disease is responsible for an estimated 2 million deaths per year, with liver cirrhosis being the 11th leading cause of death globally[11]. In terms of specific types of liver disease, viral hepatitis is a major contributor to the global burden of liver disease, and steatotic liver disease is also a growing concern, with its prevalence increasing globally in parallel with the obesity epidemic. Liver cancer is also a substantial contributor to the global burden of liver disease, representing the second leading cause of cancer-related deaths worldwide[11,12].

Contextualization of cellular senescence

Senescence was first described in 1961 by Hayflick and Moorhead, who discovered the finite proliferative capacity of normal cells in culture[13]. Six decades since then, we know that this phenomenon is due to a particular type of cellular senescence (replicative senescence) caused by the shortening of telomers after extensive proliferation[14]. However, other stimuli can trigger senescence independently of this telomeric

process, such as DNA damage, cellular stress inducers and oncogene activation[2,15].

It is important to highlight that cellular senescence elicits beneficial biological functions, including tumour suppression, regulation of embryonic development and wound healing. However, prolonged and accumulated senescence results in detrimental consequences, leading to tumour development, chronic inflammation, immune deficit and stem cell exhaustion[16].

Senescence is defined by a highly heterogeneous and complex phenotype, which can differ depending on the cellular type and the senescence inducer[17]. However, there is a certain degree of agreement regarding the key characteristics of senescent cells that represent overall senescence.

One of the hallmarks of cellular senescence is a stable cell cycle arrest, which differs from other non-proliferative cellular states such as quiescence and terminal differentiation[18]. This process is coordinated by the activation of *TP53* (coding for p53) and different cyclin-dependent kinase (CDK) inhibitors, such as p16 and p21, which results in persistent activation of the retinoblastoma family proteins and the inhibition of transcriptional activators (E2F family) that are required for cell cycle progression[19,20]. Moreover, in vitro, senescent cells undergo morphological changes, becoming flattened and enlarged, with dramatic changes in chromatin structure that can be visualized as associated heterochromatin foci[21]; however, they seem to be cell type-dependent and stimulus-dependent as they are not present in all senescent cells, such as mouse fibroblasts, as opposed to human fibroblasts[22], or during replicative cellular senescence in human fibroblasts resistant to premature $p16^{INK4a}$ activation[23].

Furthermore, senescent cells activate a complex pro-inflammatory response known as senescence-associated secretory phenotype (SASP). SASP comprises pro-inflammatory cytokines and chemokines, growth modulators, angiogenic factors and matrix metalloproteinases (MMPs). These SASP components are crucial in mediating the pathological and physiological effects associated with senescence[1,24]. These secreted factors can stimulate inflammatory and immune responses in neighbouring cells, resulting in an altered tissue microenvironment and subsequent damage to surrounding tissues, and the modulation of the phagocytic clearance of senescent cells[25–27]. SASP is induced by DNA damage and the presence of cytosolic chromatin fragments, followed by the induction of proteasomal degradation of histone methyltransferases, such as G9a and GLP, through the activation of APC/C^{Cdh1} ubiquitin ligase[28], the upregulation of LINE-1 elements[29], and the transcription of stimulator of interferon genes (STING), which promotes the production of SASP factors[30]. Remarkably, STING inhibition in human tissue and fibroblasts, and in mice, has been associated with a decrease in the pro-inflammatory phenotype of senescent cells[31].

The number and size of lysosomes in senescent cells increase[32], and this feature has been associated with the accumulation of senescence-associated β-galactosidase (SA-β-gal). Activity of this enzyme has been widely employed as a marker for identifying senescent cells through histochemical staining at a pH of 6.0 (refs. 33,34). Nevertheless, it is noteworthy that SA-β-gal staining exhibits certain limitations, including a susceptibility to generate false-positive results under specific cellular conditions, such as in vitro cell confluence or serum starvation, or within particular cell types or mutations, that show cell senescence, but do not express SA-β-gal[34], potentially leading to misleading interpretations[35]. Consequently, other methods are being used in conjunction with SA-β-gal assessment, such as lipofuscin aggregate staining, which is another characteristic of the senescent increased lysosomal content. In contrast to SA-β-gal, this approach can be applied to formalin-fixed and paraffin-embedded tissue samples while enabling co-staining with other markers, such as p16 and p21 (refs. 1,35).

Thus, as a unique marker for senescent cell detection has not been established, assessing the combination of multiple factors associated with senescence is recommended to define this particular phenotype and correctly discriminate it from other cellular states[1]. For instance, a multi-marker, three-step workflow to assess senescence has been proposed, which combines SA-β-gal or lipofuscin quantification, followed by co-staining with other known markers of senescence such as p16 or p21, and further validated with specific markers of the particular senescent context[1], such as SASP molecules of a specific senescent stimulus or cell type[36].

Box 1

Clinical effect of ageing on liver disease

Older individuals are more likely to develop liver disease[179]. During ageing, the liver undergoes a gradual decrease in volume and blood flow, coupled with increases in fat and cholesterol contents. The mass of functional liver cells declines, whereas the prevalence of polyploid nuclei and dysfunctional mitochondria rises. These alterations collectively disturb homeostasis and, consequently, liver susceptibility to injury, leading to a poor prognosis in diseases such as MASLD, viral hepatitis and ALD[6,180]. Clinical studies have shown that MASLD prevalence increases with age[7,181] being 16% higher in older patients than in younger ones, with the former having an increased risk of advanced fibrosis during MASH, among other features of severe damage[116]. The prevalence of ALD and its complications also increase with age[182], as well as the progression and complications related to hepatitis B and C infection[183,184]. Consequently, these factors contribute to an increased HCC incidence in older patients with cirrhosis following HCV cure, compared with younger ones[185]. Furthermore, the regenerative capabilities of the liver are affected by age, showing a decreased proliferation, which might contribute to decreased survival after liver transplantation in older patients[6,180]. Interestingly, age affects the clearance of CYP3A substrates, including several therapeutic agents, such as benzodiazepines or opioid analgesics, among others, and endogenous metabolic reactions, including many steroid hormones[186]. This effect influences not only drug effectiveness in older patients but also their susceptibility to adverse drug reactions or drug-induced liver injury. Accordingly, the higher prevalence and severity of liver diseases in older populations might be explained by longer exposure to detrimental factors and cellular functionality decline over time. Within this framework, it is crucial to acknowledge that cell senescence might represent a substantial underlying mechanism of liver disease pathophysiology in older people.

ALD, alcohol-related liver disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis.

Effect of senescence on liver cell phenotype

Most of the current knowledge on the role of senescence in liver cells is grounded in studies related to ageing. However, it is essential to distinguish between cellular senescence and ageing. Although cellular senescence represents a specific molecular phenomenon hallmark of ageing, the latter constitutes a complex biological process involving a multitude of changes throughout the organism, including senescence.

Hepatocytes

Senescence has an important effect on hepatocytes, particularly their function, which can negatively affect the liver's ability to perform its crucial functions, such as detoxifying the blood, producing bile and synthesizing proteins[37]. Additionally, senescent hepatocytes are subjected to metabolic changes, including higher lipid and glucose uptake, with increased expression of genes involved in cholesterol uptake and synthesis[38] and, at the same time, deregulation of glucose transporter GLUT2 and GLUT4 expression, which causes selective insulin resistance[39]. These senescent hepatocytes have been observed to be predominantly located in periportal or periseptal areas in human liver samples, in which more cell death and the subsequent regeneration occur[40,41].

Related to this, hepatocytes in the aged liver also show several morphological characteristics, such as diminished volume, accumulation of dense bodies in the cytoplasm, a decline in smooth-surfaced endoplasmic reticulum and its respective enzymes, and an increase in polyploidy[6,42].

It is believed that hepatocyte senescence in the aged liver is vital to limit the growth of DNA-damaged cells, which might eventually transform into HCC[37]. In fact, not only ageing but also liver injury stimulates hepatocyte senescence[43]. Whereas acute liver damage results in the transient presence of senescent hepatocytes with a low probability of progressing into malignant cells, chronic injury, which increases the chance of DNA damage, necessitates a sustained and heightened degree of hepatocyte regeneration[43]. This sustained regeneration can lead to the proliferation of DNA-damaged hepatocytes, which can develop to cancerous cells and can be detrimental to liver function[43]. More specifically, senescent hepatocytes accumulate in the liver of patients with chronic liver disease (CLD), and studies have shown that the degree of accumulation is correlated with fibrotic stage[44–46], increased expression of ACTA2 (also known as α-smooth muscle actin or α-SMA)[47] and activation of HSCs[47]. This link can be explained by the effect of senescent hepatocytes over HSCs in vitro, inducing their activation, followed by the upregulation of inflammatory and fibrogenic pathways[47,48]. Specifically, nuclear factor erythroid 2-related factor 2 has been reported to be a key molecule involved in this process, at least in senescent hepatocytes induced by a high content of free fatty acids[48]. Also, other in vitro studies have shown that senescent hepatocyte SASP molecules, specifically the secretion of IL-8 and IL-6, promote the recruitment of pro-inflammatory macrophages that might contribute to the inflammation of the tissue and the progression of liver disease[49].

On the other hand, it is not only direct hepatocyte damage that can induce senescence, but also the sustained hepatocyte turnover during regeneration promotes the shortening of telomeres, which, in turn, activates senescence. This shortening might increase cell death, further exacerbating liver damage, as shown in telomerase-deficient mice[50]. One of the mechanisms being studied as key in the diminished regenerative response of older liver is sirtuin 1 (SIRT1). This protein is known to be critical in the regulation of ageing; specifically in the liver of old mice, SIRT1 is repressed by the CEBPB–HDAC1 complex, which inhibits its expression, reducing the liver proliferative capabilities[51].

An additional study demonstrated that in the liver of aged mice, *Foxo3* and *Foxm1* mRNA expression was downregulated through the AKT and retinoblastoma pathways, respectively. Consequently, this downregulation reduces the levels of reactive oxygen species (ROS) scavenger proteins, notably SOD2, resulting in the concomitant accumulation of ROS, ultimately inducing DNA damage and establishing a state of cellular senescence[52].

Senescent hepatocytes might help prevent HCC by secretion of various SASP cytokines and chemokines. These molecules induce an immune-mediated clearance of senescent premalignant cells. However, the malfunctioning or escape of this senescent surveillance can result in the survival of these cells, which can further replicate leading to the development of HCC[53,54]. This process is explained in more detail in the section 'Hepatocellular carcinoma'.

Although there are many senescence markers and SASP components, some have been identified as being pivotal in hepatocytes. For instance, SMP30 (also known as regucalcin) is a senescence marker in hepatocytes that suppresses cell proliferation[55]. CEBPA is another transcriptional factor pivotal in liver function; it directly interacts with CDK2 and CDK4, inhibiting cell proliferation[56,57]. Still, further research is necessary to identify biomarkers of senescence, specifically in the different cell types of the liver.

Senescent hepatocytes have also been suggested to induce senescence of nearby cells through a process known as senescence-induced bystander senescence, which is discussed in the section below 'Importance of SASP in hepatic paracrine communication'[58,59].

In summary, hepatocyte senescence has a dual role: it might protect against malignancies by limiting the growth of damaged cells, but it can also impair liver function and the function of neighbouring cells, and therefore it has a crucial role in the pathogenesis of liver diseases (Box 2).

Liver sinusoidal endothelial cells

Initial studies in the field of ageing and LSECs suggested that there are no major age-related changes in the liver endothelium[60]. However, current studies have demonstrated the marked alterations in the structure and functions of LSECs during the ageing process[61–64], which are probably interconnected with an increase in cell senescence[65] (Box 2).

Age-related morphological changes in LSECs are characterized by a reduction in porosity and the number of fenestrations, endothelial thickening and the deposition of perisinusoidal basal lamina, resembling capillaries found in other non-fenestrated vascular beds[66]. These changes are called pseudocapillarization of the sinusoidal endothelium, which is distinct from the more pronounced capillarization effect observed in LSECs after chronic liver injury[67]. LSEC pseudocapillarization has been described in several species, including humans, rats and mice[61,68,69]. Loss of fenestrations, which serve as windows for the uptake of chylomicron remnants and insulin, can result in hyperlipidaemia and hepatic insulin resistance in rats[70]. These morphological changes are also accompanied by functional alterations, including a moderate pro-inflammatory state, increased mitochondrial oxidative stress, and modulation of the expression of genes related to senescence, such as *Tnfα*, *Il1* and *Il6*, among others[61,62,71]. These alterations might heighten the susceptibility to liver injury or disease development in the older population by exacerbating the dysfunction of LSECs. Indeed, preclinical studies have demonstrated that the sinusoidal endothelium of aged rats exhibits greater vulnerability to acute insults,

such as ischaemia–reperfusion injury, than that of their younger counterparts[72].

Moreover, with age-associated senescence, typical markers of the healthy LSEC phenotype, such as VEGFR2 and FCGR2B (also known as CD32B), are lost, and this is accompanied by diminished vasodilatory capacity[61]. Similar outcomes were found in LSECs from cirrhotic aged rats, in which more pronounced deregulation of sinusoidal dedifferentiation markers, such as KLF2 and FCGR2B, was observed compared with their younger cirrhotic counterparts[64]. This deregulation was concomitant with a decrease in porosity and frequency of fenestrations[64]. These results reinforce the importance that senescent LSECs might have in the increased prevalence and susceptibility to liver diseases in older patients.

P16^{INK4a} and p19^{ARF} (p14^{ARF} in humans) proteins have been proposed as regulators of in vitro replicative senescence of LSECs[73,74]. Depletion of *p19*^{Arf} in mice enabled LSEC to evade senescence and proliferate while retaining their endothelial characteristics[73]. This notion was supported by the increased *p16* gene expression in LSEC in ageing mice and rats[61,74]. It has been suggested that CGAS–STING signalling, a cytoplasmic DNA-sensing pathway in cellular senescence, could be an interesting pathway to promote senescence in hepatic endothelial cells[75]. Hepatic C-KIT (also known as CD117), a receptor tyrosine kinase used to discriminate and sort LSECs from the pericentral region of the liver[76], has been found to be downregulated during ageing reprogramed liver endothelial zonation, and the researchers of the study suggested that abrogation of endothelial *Kit* in mice triggered cellular senescence, which could disturb LSEC homeostasis[71].

Although the specific stimuli or causes promoting LSEC senescence are not yet well understood, they are probably similar to those affecting other endothelial cells. Endothelial cells, including LSECs, are constantly exposed to circulating blood that contains various substances capable of contributing to senescence, such as oxygen, glucose, lipids, amino acid metabolites, immune cells, ROS and inflammatory cytokines[77]. Additionally, haemodynamic forces generated by blood flow, such as shear stress and high or pulsatile pressure, can also induce senescence[77]. Regarding haemodynamics, senescent endothelial cells exhibit reduced plasticity when adapting to changes in haemodynamic conditions. Specifically, they have limited ability to alter their shape and orientation in response to blood flow, caused by the increased adhesion to the substrate and higher contractibility induced by senescence, which are mechanisms necessary to prevent dangerous endothelium permeability and denudation of the underlying substrate[78]. Although these characteristics have not been specifically studied in senescent LSECs, they align with the observed microcirculatory dysfunction and structural abnormalities in the hepatic sinusoid that appear in CLD[9] and could partially explain the higher susceptibility in the older population.

During ageing, the scavenger functions of LSECs are compromised, as evidenced by a reduction in their endocytic capacity[79,80]. Interestingly, it is believed that the dynamics of scavenger capacity throughout an organism's lifespan could be a crucial mechanism promoting LSEC senescence. For instance, in mice, owing to the loss of endocytic capacity of senescent hepatocytes, LSECs overcompensate, leading to an increased intake of oxidizing molecules, which promotes their senescence. As senescence progresses, heterochromatic silencing occurs, suppressing various scavenger receptors and endocytosis-related genes[74]. Certainly, dysfunction in endocytosis within aged LSECs leads to the systemic accumulation of circulating products. This accumulation poses an increased risk of various age-related diseases, such as the formation of atherosclerosis[81].

Box 2

The hepatic sinusoid and senescence

Hepatic sinusoidal cells, including hepatocytes, liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and Kupffer cells, are susceptible to undergoing senescence during ageing or liver disease[4]. Senescent hepatocytes undergo morphological changes, including a reduction in cellular volume and an increase in nuclear polyploidy[6,52]. These changes are accompanied by an uptake of lipids and glucose.[38] The increased secretion of senescence-associated secretory phenotype (SASP) by senescent hepatocytes recruits pro-inflammatory macrophages and liberates cell proliferation inhibitors, contributing to senescence expansion through the liver environment[49]. Senescence of LSECs leads to pseudocapillarization, reduced endocytic and vasodilatory capacity, decreased plasticity in blood flow, and increased production of mitochondrial oxidative stress[61,62,66,67].

Additionally, LSECs have a role in regulating immune surveillance, facilitating the clearance of senescent cells[84]. Senescent HSCs exhibit beneficial effects by reducing the degree of their activation and matrix deposition, but increasing the production of matrix metalloproteinases, which aid in the resolution of fibrotic scars[92]. Senescent HSCs also acquire a pro-inflammatory phenotype, promoting immune surveillance[93]. With ageing, the population of Kupffer cells increases and their phagocytic capacity is enhanced[101], and the production of IL-6, known as a senescence inducer, is also increased[61,103]. Senescent hepatic sinusoidal cells can be identified through the detection of well-established senescence features, including cell cycle arrest (as indicated by upregulation of p21 and p16 cell cycle inhibitors), oxidative damage (as demonstrated by increased levels of reactive oxygen species), metabolic changes (such as the accumulation of senescence-associated β-galactosidase), the presence of senescence-associated heterochromatin foci, and the manifestation of a SASP[1,33–37].

Senescent LSECs exhibit decreased expression of diverse angiocrine receptors and factors, such as stabilin 2, CD32B and VEGFR2, and decreased nitric oxide availability. These changes can affect hepatic functions, including steatosis, metabolism, vascular tone and regeneration[82]. Indeed, Duan and colleagues found that, in mice after partial hepatectomy, LSEC senescence induced by shear stress through the Notch signalling pathway interfered with liver regeneration[83]. In rats, the aforementioned alterations affected vascular functionality, the maintenance of other liver cells and the development of clinical complications associated with liver injury, including increased hepatic vascular resistance and portal hypertension[61].

Despite the pathological implications associated with LSEC senescence, it is noteworthy that this biological process occurs naturally within the liver and, when appropriately regulated, has an essential role in maintaining hepatic homeostasis[3]. SASP-primed LSECs have been characterized as centres that organize and amplify SASP activity. This phenomenon drives immune-mediated surveillance of senescent cells and facilitates their clearance in the liver, as shown in LSEC

from humans and mice[84]. Soluble factors released by senescent cells stimulate the activity of NF-κB in LSECs, leading to the recruitment of STAT1-activated subset of $CD4^+$ T lymphocytes and subsequently influencing the outcome of senescence surveillance[84]. Surprisingly, senescent LSECs might not be replaced by non-senescent cells, a finding quite relevant for vascular structure and function in ageing. Indeed, in this study, genetic ablation of *Cdkn2a*-high senescent cells in mice disrupted the blood–tissue barrier, activated HSCs and promoted liver and perivascular tissue fibrosis and deterioration of health[74]. Nevertheless, further studies are required to determine properly whether the elimination of senescent LSECs could be beneficial or detrimental in the aged liver or CLD.

Hepatic stellate cells

HSCs seem susceptible to undergoing replicative senescence, as they cannot be maintained in vitro during multiple passages[85]. Supporting this idea, aged-related telomere attrition in human liver donors was shown to be restricted to Kupffer cells and HSCs[86]. However, it should be noted that the extent of telomere shortening and the cell types affected is contingent upon the stimulating factors involved. In human cirrhosis; for instance, telomere shortening is more pronounced in hepatocytes rather than in HSCs[87].

Mouse and rat models of ageing have demonstrated an increment in the number of HSCs, accompanied by intracellular accumulation of lipids[61,88]. Notably, this contradicts what is observed during liver dysfunction, in which the loss of lipid droplets is a characteristic of HSC activation[89]. In this regard, our group has shown a relevant increased protein and gene expression of HSC activation markers, including Acta2 and platelet-derived growth factor receptor-β, together with inflammatory, pro-oxidant and senescence markers, indicating a partial activation of HSCs during ageing[61]. Moreover, during cirrhosis, the HSCs of aged rats showed an increased deposition of extracellular matrix (ECM) compared with younger animals[64], indicating the importance of age in CLD development (Box 2).

Indeed, activation of quiescent HSCs due to liver injury or inflammation induces their transdifferentiation into myofibroblast-like cells. This transformation is accompanied by cell proliferation, induction of inflammation and ECM remodelling, with activated HSCs becoming the primary source of ECM components in damaged liver tissues[90]. Activated human HSCs that undergo replicative senescence in vitro switch from a fibrogenic to a pro-inflammatory phenotype[91]. In the cirrhotic liver, an accumulation of senescent HSCs has been described, which seems necessary to restrict fibrosis progression. This accumulation correlates with the increase in fibrotic tissue when senescence induction is reduced in the absence of TP53 (also known as P53)[92]. Moreover, senescent HSCs release factors, such as IFNγ and IL-6, which induce a shift to a pro-inflammatory type in liver-resident macrophages, promoting immune surveillance and facilitating the effective clearance of senescent cells. Conversely, HSCs lacking functional p53 secrete specific factors that facilitate the polarization of macrophages towards a tumour-promoting phenotype through the secretion of IL-33, therefore enhancing the proliferation of premalignant cells[93–95]. Hence, the senescent programme plays a crucial role in the liver by regulating the excessive activation of HSCs, facilitating liver regeneration and the regression of liver fibrosis.

Furthermore, other studies have focused on the role of senescent HSC as inflammatory and immune response regulators. In fact, senescent HSCs show an increase in surface expression of natural killer cell-activating ligands, promoting their own elimination by the immune system[96]. The cytokines IL-10 and IL-22 were described in rats and mice, respectively, as senescence inducers in activated HSCs via the STAT3–P53 pathway, ultimately ameliorating liver fibrosis[97,98].

Finally, the induction of senescence in HSCs in mice resulted in the downregulation of proteins associated with the formation of fibrotic scar, such as collagens I, III and IV, and fibronectin. In addition, senescent HSCs exhibited an increase in the expression of MMPs that possess fibrinolytic activity. These changes in protein expression patterns contribute to the remodelling of the ECM and the breakdown of fibrin, potentially facilitating the resolution of fibrosis[92].

Hepatic macrophages

Kupffer cells represent between 80% and 90% of the liver-resident macrophages and are located in the lumen of the hepatic sinusoid[99]. In a healthy liver, Kupffer cells have an anti-inflammatory and phagocytic phenotype, clearing immunoreactive material, which arrives at the sinusoid from the circulation, and dead cells in the liver and bloodstream. However, upon liver damage, Kupffer cells polarize, resulting in a pro-inflammatory response characterized by the secretion of cytokines and ROS. The Kupffer cell pro-inflammatory response initially offers protective mechanisms to the damaged liver but, when sustained over time, switches towards a damaging response that further aggravates liver disease[100]. Thus, Kupffer cells are major players in the immune and inflammatory response of the liver, whose functions are altered during senescence (Box 2).

Preclinical studies have found that aged livers exhibit a higher number of Kupffer cells than younger counterparts, accompanied by an increased phagocytic activity[101] and lower levels of autophagy[102] (Box 2). The altered autophagy of aged Kupffer cells is thought to predispose the liver to a higher degree of inflammation after liver injury[102]. Regarding inflammation, Kupffer cells in aged rats secrete more IL-6 (refs. 61,103), which promotes hepatocyte senescence and aggravates alcohol-related liver senescence[103]. This process seems key in the early stages of ALD by protecting hepatocytes against apoptosis and steatosis; however, it aggravates liver disease in more advanced stages[103].

Although senescent hepatic macrophages have not been extensively studied, research focusing on resident macrophages in other tissues shows evident deregulation of autophagy, phagocytic clearance of substances and antigen presentation capacity[104]. Owing to the lack of knowledge of senescent Kupffer cells, further research is needed to understand and find novel therapeutic strategies to reduce the pro-inflammatory response that senescence might induce.

Importance of SASP in hepatic paracrine communication

As described above, SASP encompasses a multitude of factors released by senescent cells, including cytokines, chemokines, interleukins and growth factors, such as IL-1, IL-6, C-C motif chemokine ligand 2 (CCL2) and insulin-like growth factor, among others[24]. These secreted molecules exert diverse paracrine effects on neighbouring cells, including immunomodulation, pro-tumorigenicity, senescence reinforcement and modulation of the tissue microenvironment[24]. Thus, SASP has a crucial role in mediating cellular communication and influencing various pathological processes.

SASP influences the immune response by activating senescence surveillance, facilitating immune cell recruitment and eliminating senescent cells. Conversely, SASP can also stimulate cell proliferation and contribute to malignant transformation, promoting

epithelial-to-mesenchymal transition, invasiveness and abnormal tissue morphology. Moreover, this particular molecular signature can further enhance senescence either by reinforcing the senescent phenotype in cells already committed to it or by inducing senescence in healthy cells through the secretion of various interleukins, a phenomenon known as the bystander effect[105,106], which might explain, partially, why in the liver, senescent hepatocytes tend to cluster together[59].

IL-1 and IL-6 are the most abundant pro-inflammatory cytokines in the SASP[107], whereas in the liver, as discussed above in the section 'Hepatic stellate cells', IL-22 induced HSC senescence in mice, protecting against fibrosis[98]. Also, macrophage-dependent paracrine transforming growth factor-β (TGFβ) signalling intensified injury-induced senescence in the mouse liver by causing healthy and non-injured hepatocytes to undergo senescence[108]. Another mechanism by which cells can induce senescence in neighbouring cells is through direct cell–cell contact, specifically via gap junctions. In vitro, this contact leads to the production of ROS in the target cell, subsequently causing DNA damage[59]. A different mechanism that works indirectly is through the recruitment of neutrophils by senescent cells, which in mice promoted ROS production in target cells, also via cell–cell contact, inducing telomere dysfunction and senescence in the liver[109].

Through the process known as immune-mediated clearance, senescent hepatocytes are eliminated from the liver, which is thought to be induced, at least partially, by IL-1 signalling — the in vitro inhibition of its receptor reduces senescence surveillance of oncogene-induced senescent hepatocytes[25]. In vivo, the immune surveillance of senescent pro-oncogenic hepatocytes is dependent on Kupffer cells and macrophages; however, senescent hepatocytes, through the secretion of CCL2, recruit immature myeloid cells, which have an immunosuppressive phenotype that inhibits tumour and senescence clearance permitting the growth of HCC[53].

Although the SASP is key to promoting cell senescence, it can also influence the risk of developing HCC. For instance, the inactivation of *Tp53* in mouse HSCs promoted the secretion of factors that induce the polarization of macrophages into tumour-promoting cells, further leading to the proliferation of premalignant cells[93]. Moreover, in mice, it was shown that certain molecules derived from the gut microbiota can enter the enterohepatic circulation, reaching the hepatic sinusoid, where they induce the senescence of HSCs, which, via the SASP, secrete pro-inflammatory cytokines and tumour-promoting factors that can promote HCC development[110,111]. Thus, in the initial stages of tumour growth, the SASP induces cell senescence and immune surveillance, protecting against malignancies, whereas, in more advanced stages, SASP can directly contribute to tumoural invasion and survival of metastatic cells[112].

In summary, understanding the SASP is crucial for addressing liver diseases, as it influences the immune response, the liver microenvironment and tumour development. Unravelling the SASP complexities would aid in developing targeted therapies that could either reinforce or regulate particular effects of cell senescence in the liver, improving patient outcomes and fostering a better understanding of liver pathologies.

Role of senescence in liver diseases

Steatotic liver disease

Ageing is key in the development of MASLD and MASH (Fig. 1). Studies have revealed its effects on different vital pathways in the liver, including lipid metabolism, mitochondrial dysfunction and autophagy[113]. Besides the alteration in the balance between fatty acid oxidation and de novo lipogenesis in the liver, other alterations associated with age, such as insulin and leptin resistance, the decrease in growth hormone and adiponectin, hypercholesterolaemia, DNA damage, accumulation of ROS and telomere shortening, also contribute to a higher risk of MASLD in the older population[114,115]. In accordance, older patients also have a higher risk of developing MASH and advanced fibrosis than younger patients[116].

In relation to this, ageing causes fat redistribution in the body. More specifically, lipids get transferred from the subcutaneous adipose tissue to the intra-abdominal visceral depots and other ectopic tissues, including the liver and the muscle, which increases the risk of metabolic diseases[117]. Similarly, aged mice also showed higher fat accumulation in the liver, caused by a decrease in mitochondrial function[118] and modifications in their transcriptomic profile associated with genes involved in steatosis. However, when *Cisd2*, which mediates lifespan in mice, was upregulated, the dysregulation of genes involved in lipid metabolism was attenuated[119]. In another preclinical study, when hepatocyte senescence was induced, it also promoted liver steatosis, whereas eliminating those senescent cells reduced lipid levels in the liver[120]. Aged mice fed a high-fat diet (HFD) showed an increased inflammatory response in the liver, caused by an increased percentage of pro-inflammatory macrophages and tumour necrosis factor; however, when compared with their younger counterparts, the investigators found no differences in fat accumulation caused by age[121].

The link between cell senescence and a higher susceptibility to MASLD can be partly explained by the mitochondrial dysfunction that arises with senescence, including impaired oxidative phosphorylation and increased ROS generation[38,122]. This dysfunction impaired the ability of mouse hepatocytes to effectively catabolize lipids, leading to their accumulation in the cytosol[120]. More specifically, preclinical studies showed that senescence disturbs glucose and lipid metabolism, inducing mitochondrial dysfunction and ROS, which disrupts fatty acid oxidation, causes the accumulation of lipids in the liver and steatosis, which can further progress into MASH[113,123]. Moreover, in another study, hepatocytes from 70 patients with MASLD had shorter telomeres and higher gene expression levels of *CDKN1A* (coding for p21) than those from 60 healthy matched donor livers, which correlated with fibrosis stage and adverse liver-related outcomes[124]. Chronic hyperinsulinaemia, as observed in obesity and metabolic syndrome, can also predispose hepatocytes to become senescent through the expression of p53 and p21 protein levels. This process is induced by insulin as inhibiting its signalling pathway protected hepatocytes against senescence[125]. Similarly, as observed in mice and HepG2 cells, senescent hepatocytes also have a higher lipid and glucose uptake, with an increased expression of genes involved in cholesterol uptake and synthesis, which might explain the increased susceptibility of older patients to develop metabolic liver disease[38]. On the other hand, preclinical and in vitro studies have also shown that the increase in fatty acids in the liver and its consequent upregulation in fatty acid β-oxidation induces a higher generation of ROS in the mitochondria, promoting telomere erosion, direct DNA damage and a decrease in electron transport chain activity, with the latter activating pathways involved in senescence[126]. Finally, an increase in hepatocyte fat accumulation in mice was accompanied by an increase in markers of senescence[122].

Thus, it seems that senescence causes increased lipid accumulation in the liver, but lipids themselves are also capable of inducing hepatocyte senescence, which might cause an autoregulatory loop that further aggravates both conditions.

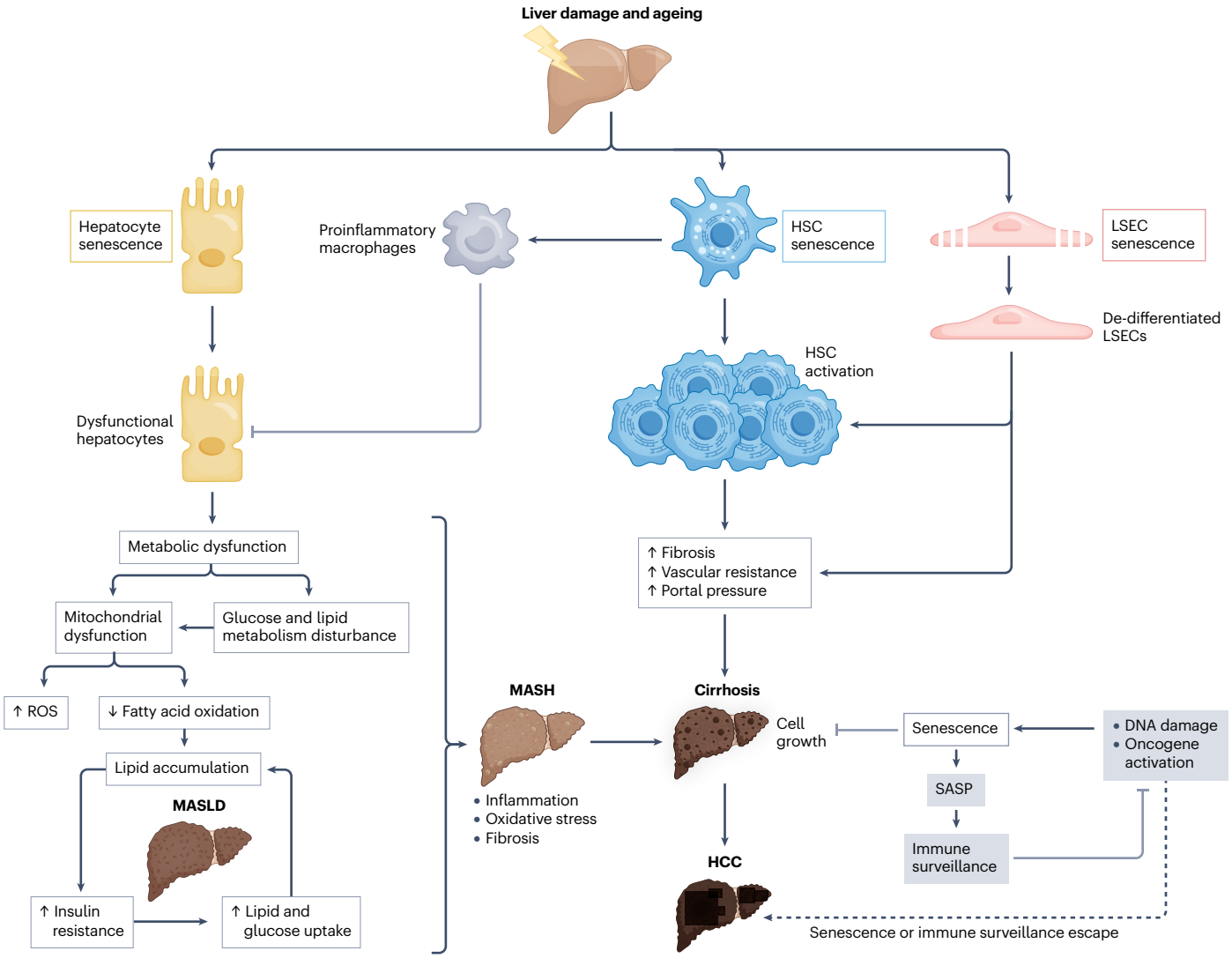

Fig. 1 | Role of senescence in liver diseases. Liver damage and ageing induce senescence in the different cell types of the liver by a combination of inflammation and telomere attrition caused by DNA damage and the increased cellular turnover necessary for liver regeneration. Hepatic stellate cell (HSC) senescence is a mechanism induced to control excessive fibrosis caused by activated HSC, which also recruits M1-polarized macrophages that promote immune surveillance and facilitate the effective clearance of senescent cells. On the other hand, senescence of liver sinusoidal endothelial cells (LSEC) induces their pseudocapillarization, which leads to the secretion of inflammatory molecules, altering vascular functionality, and inducing the development of other clinical complications such as increased hepatic vascular resistance and portal hypertension. At the same time, hepatocyte senescence not only contributes to liver cirrhosis by activating HSCs and therefore also to an increase in fibrosis, but also, owing to changes in their phenotype, leads to higher susceptibility of hepatocytes to metabolic dysfunction as a result of impaired mitochondrial function and disturbance in glucose and lipid metabolism, that in turn leads to accumulation of reactive oxygen species (ROS) and lipids, which is associated with insulin resistance and higher uptake of lipids and glucose by hepatocytes. These alterations partly cause liver metabolic dysfunction-associated steatotic liver disease (MASLD), which, over time, can further progress to metabolic dysfunction-associated steatohepatitis (MASH) and cirrhosis. Although cell senescence is a key protective mechanism against hepatocellular carcinoma (HCC) by detecting DNA damage and oncogene activation, and inducing immune surveillance that clears premalignant cells, some of these cells can escape senescence and immune surveillance and ultimately develop HCC. SASP, senescence-associated secretory phenotype.

Viral hepatitis

In the context of viral hepatitis, senescence can occur in hepatocytes and other liver cells in response to the presence of the virus, and this is independent of patient age. Virus infection causes cellular stress, leading to the activation of senescence pathways and the secretion of a variety of pro-inflammatory cytokines, growth factors and proteases, which can contribute to liver injury, inflammation and fibrosis that are hallmark features of liver disease (Fig. 1).

More in detail, diverse clinical studies have shown that senescence has a role in the development and progression of liver disease in various forms of viral hepatitis, including hepatitis B and C[41,46,127,128]. The presence of senescent cells has been associated with increased

liver injury, fibrosis and reduced liver function in patients with these conditions[41,46,128]. In a study including 91 patients with hepatitis B virus (HBV) infection, virus infection was associated with an increase in hepatocyte senescence, which correlated with liver fibrosis[46]. Furthermore, current knowledge shows that insufficient clearance of both senescent and precancerous hepatocytes, coupled with compromised immune surveillance due to immunosenescence triggered by chronic HBV infection, can contribute to hepatocarcinogenesis[127].

Chronic hepatitis C virus (HCV) infection and the consequent liver inflammation accelerate the process of telomere shortening, leading to a state of replicative senescence that predisposes individuals to HCC[41]. Similar to HBV infection, in a study in 61 patients with HCV infection and 22 age-matched healthy individuals, the level of cellular senescence correlated with the progression of liver fibrosis in the patients. Interestingly, senescent markers were more prominent in non-parenchymal fibrotic tissue which was attributed to an increased proportion of intrahepatic senescent T cells[128]. The diminished activity of T cells in HCV infection might create a predisposition to the occurrence of HCC, as T cells have a crucial role in orchestrating the clearance of senescent premalignant hepatocytes[54].

Finally, hepatitis delta virus (HDV) infection has been correlated with premature immunosenescence in patients characterized by cellular ageing of immune cells and compromised T cell function[129]. Additionally, elevated protein levels of the senescence marker CD57 were observed in $CD8^+$ T cells in 49 patients with HDV infection, indicating a potential association between HDV infection and immune cell senescence[129]. These findings suggest a possible link between HDV infection, immune cell senescence and the development of liver cancer.

Liver fibrosis and chronic liver disease

In the context of persistent insults, cells undergoing damage exhibit a heightened susceptibility to entering a senescent state, as evidenced in numerous chronic diseases in preclinical and clinical studies[18]. The accumulation of senescent cells within affected tissues substantially affects disease progression by exerting an influence over the microenvironment and disrupting tissue homeostasis[18,26] (Fig. 1).

Damaged livers from two cohorts of patients with ALD (one of 42 patients and one of 77 patients) exhibited a marked increase in the senescent hepatocyte population during advanced disease[44], which indicates the important role of senescence during disease development. This role is supported by the telomere attrition reported in 52 patients with cirrhosis, which was found to be correlated with progression of liver fibrosis[87]. Accelerated telomere degradation has been suggested to be a factor leading to end-stage organ failure in chronic diseases characterized by heightened cellular turnover, such as liver cirrhosis[130]. In a clinical study in 69 patients with cirrhosis, loss-of-function mutations in the telomerase reverse transcriptase (TERT) and telomerase RNA component genes were found to be implicated in familial liver abnormalities[131]. In this regard, in a mouse model deficient in telomerase specifically lacking the telomerase RNA gene, telomere dysfunction was associated with impairment in liver regeneration and an accelerated progression of liver cirrhosis following chronic liver injury[50].

As described above, the immune response associated with CLD might contribute to senescent cell accumulation by impairing the clearance mechanism or further inducing senescence differentiation[3]. Indeed, M2-polarized macrophages trigger hepatocyte senescence in vivo and in vitro via the release of IL-6 and enhance alcohol-induced hepatocyte senescence while decreasing alcohol-induced hepatocyte apoptosis[103]. Liver-resident macrophages also participate in senescence spreading to bystander cells through TGFβ1 ligand[108].

Injured livers exhibit impaired hepatocellular function, which can probably be attributed to metabolic alterations occurring in senescent hepatocytes. As explained above, dysregulation of crucial genes involved in glucose, lipid and protein metabolism was described in vitro in hepatocyte senescence[132]. Moreover, the expression of bilirubin transporters, MRP2 and MRP3, is altered in senescent hepatocytes. In particular, decreased expression of MRP2 in senescent hepatocytes in vitro, coupled with absence of MRP2 protein in p21-positive hepatocytes, indicates a reduced ability to transport conjugated bilirubin into the biliary canaliculi. Conversely, increased expression of MRP3 in senescent hepatocytes might serve as a compensatory mechanism, augmenting the transport of conjugated bilirubin into the hepatic sinusoid[133]. This finding could explain the development of jaundice (conjugated bilirubinaemia) in advanced liver disease.

In a study in patients with ALD, including 77 patients with ALD cirrhosis, a spatial association was found between senescent p21-positive hepatocytes and activated HSCs[44], supporting the notion that hepatocyte senescence, activation of HSCs and fibrosis are linked. As mentioned above, senescence of activated HSCs facilitates tissue repair and resolution of liver fibrosis while limiting scarring progression; however, the positive effect of HSC senescence during fibrosis and CLD might depend on the inducing stimuli. Different types of induced senescence in human HSCs have been shown to enhance phosphorylation of ERK1 and ERK2, and to induce expression of *ANGPTL4*, *Il8*, and *PF4V1* genes, which have been associated with the progression of liver disease to HCC[134]. In addition, in a study characterizing the hepatic microbiome disturbance during the progression of liver cirrhosis, 46 patients with HCC showed intrahepatic dysbiosis, which enhanced the production of SASP by HSCs. This, in turn, activated the NLRP3 inflammasome complex and the release of various inflammatory factors in the liver. These factors contributed to the progression of liver cirrhosis to HCC[135]. Considering this, it is prudent to contemplate strategies that target the elimination of senescent HSCs after therapy-induced senescence.

Hepatocellular carcinoma

HCC is the predominant form of primary liver malignancy, accounting for approximately 75–85% of all cases of liver cancer. This type of cancer is primarily associated with risk factors such as HBC and HCV infections, excessive alcohol consumption and obesity, which substantially contribute to its pathogenesis[136].

Senescence has a pivotal role in HCC owing to its importance in tumour suppression and progression. The consequences of hepatic senescence can differ depending on the stage of the tumoural process. For instance, during early stages, the senescence of damaged hepatocytes prevents their transformation into malignancies, whereas senescence in HSCs mainly controls and restricts their activated, proliferative and pro-inflammatory state[137]. However, in preclinical models of advanced HCC, senescence contributes to its progression, altering the tumour microenvironment through SASP[43,138].

Induction of senescence is one of the primary mechanisms through which the organism prevents tumorigenesis, acting as a barrier against the development and progression of HCC. Senescence inhibits the growth of damaged or malignant cells that could potentially give rise to a tumour, mainly through p16 and p53 activation, arresting the

cell cycle[112]. Moreover, it activates immune surveillance through the SASP, which enables the elimination of premalignant cells by signalling immune cells, as shown in a preclinical study[54].

Nevertheless, cancer cells can acquire the ability to escape senescence, enabling them to continue proliferating and contributing to tumour growth[54,137]. The frequent presence of p16 and p53 within premalignant and benign neoplastic tissues has been observed in patients, concomitant with their inactivation in advanced malignant tumours. This finding underscores the pivotal roles these molecular entities have in the progression of tumorigenesis[112].

In a study assessing the impact of liver inflammation, hepatocytes from 33 patients with HCC exhibited higher protein expression levels of senescence markers, resulting from DNA damage, than hepatocytes from 37 patients with cirrhosis and without HCC[139]. In fact, studies have demonstrated a correlation between hepatocyte senescence, SASP and hepatocarcinogenesis[138]. The extent of injury also has a crucial role in senescence induction and HCC. Severe acute liver injury prompts hepatocytes to enter a senescent state as a protective measure to preserve the liver microenvironment and homeostasis. Conversely, in mice, hepatocyte senescence is not activated to the same level during chronic injury, which increases the risk of malignant transformation[43]. Furthermore, although premalignant hepatocytes regularly undergo senescence triggered by DNA damage or activation of oncogenes[54], the induction of a pro-regenerative environment following liver injury in mice stimulated hepatocyte growth, posing a potential risk for HCC development[140].

Although senescence is generally considered a protective mechanism against HCC, studies indicate that it might also have a role in its initiation and progression. For example, in a study characterizing the hepatic microbiome, patients with HCC exhibited a decreased microbial diversity in the gut, and specific changes in microbial composition led to the secretion of specific molecules that induced a senescent–secretory phenotype in HSCs. The SASP of these HSCs, which included IL-1β, IL-6 and p21, among others, in turn, promoted HCC progression through the activation of inflammation[135]. More specifically, studies in mice demonstrated that deoxycholic acid, a gut microbiota-derived compound, activates senescence in HSCs, thereby promoting tumour development in the liver via the release of pro-inflammatory cytokines, such as IL-6, Gro-α and CXCL9 (ref. 111). Additionally, lipoteichoic acid, a cell wall component of gram-positive bacteria, induced the secretion of IL-33 by HSCs and promoted HCC in mice[94]. Regarding the immune response, hepatic macrophages activated by SASP from senescent hepatocytes also contribute to HCC development by fostering a pro-tumoural microenvironment in the mouse liver[138], and although senescence surveillance mediates the clearance of premalignant hepatocytes, once HCC is established, the SASP can inhibit the maturation of recruited myeloid cells involved the clearance of premalignant cells, ultimately promoting tumour growth[53].

Senotherapies as treatments for liver diseases

The persistent inflammation and disturbances during CLD, and the delicate balance between regeneration and tissue extinction, trigger cellular senescence, which is fundamental to disease progression. Thus, interventions targeting senescence, known as senotherapeutics, are potential therapies for liver disease. Senotherapeutic strategies can be broadly classified into two categories: pharmacological agents known as senolytics, which target and eliminate senescent cells, and senomorphics, such as SASP inhibitors, which aim to counteract the harmful cell-extrinsic effects of senescent cells and mitigate the pro-inflammatory secretory phenotype associated with this phenomenon (Fig. 2).

Senolytics

The first senolytics identified were a combination of dasatinib and quercetin, discovered through a hypothesis-driven, bioinformatics-based investigation[141]. Dasatinib is a FDA-approved tyrosine kinase inhibitor, and quercetin is an antioxidant flavonoid commonly found in various fruits and vegetables. In a mouse model of MASLD, the combination of dasatinib and quercetin depleted senescent hepatocytes, reducing hepatic steatosis[120]. This treatment combination showed promising results in limiting HSC SASP and tumour progression in mice driven by FBP1 loss[142].

Navitoclax (also known as ABT-263) is an inhibitor of anti-apoptotic proteins belonging to the BCL2 family that induces apoptosis and exhibits potent senolytic effects in ageing animal models and in certain types of senescence in vitro[143]. In a preclinical model of acute liver failure, navitoclax could eliminate senescent cells in vivo, alleviate SASP effects, and improve mitochondrial function[144]. Moreover, the apoptotic effects of navitoclax were enhanced synergistically by combining it with either genetic or pharmacological inhibition of *TUBB4B*, a recently discovered gene involved in cell proliferation and survival, in MASLD-associated HCC[145].

The forkhead box protein (FOXO4) is highly expressed in senescent cells and prevents cell death by binding and sequestering p53. FOXO4–D-retroinverso (FOXO4-DRI) is a peptide with senolytic activity, capable of interfering with the FOXO4–*TP53* interaction, allowing p53 to translocate to the cytosol and induce apoptosis[146]. This peptide was able to counteract chemotherapy-induced senescence and the associated weight loss and liver toxicity in mice[146].

Heat shock protein 90 (HSP90) inhibitors have also demonstrated senolytic activity[147]. HSP90 proteins have a crucial role in cellular processes by interacting with co-chaperones to ensure proper folding, stabilization, or, if necessary, degradation of client proteins[148]. One such inhibitor, 17-AAG, was utilized to inhibit the progression of MASLD in HFD-fed mice through the hepatic accumulation of so-called albumosomes — spherical shell-like structures in the cytoplasm formed by prefolding albumin — which can trap prefolding carnitine palmitoyltransferase 2 thereby maintaining mitochondrial homeostasis from exhaustion[149].

Notably, it is crucial to acknowledge that these interventions lack selectivity towards senescent cells, potentially resulting in adverse effects, including abnormal embryo development, dysregulated wound healing, and the promotion of tumorigenesis. Thus, current and future studies on senolytics must focus on explicitly targeting senescent cells and, perhaps, the cell type of interest, depending on the disease or aetiology.

Finally, it is crucial to reinforce the concept that the accumulation of senescent cells in aged and diseased tissues might be attributed to a decline in immunosurveillance. As a result, enhancing or restoring the immune system's capacity to selectively eliminate senescent cells is another approach with the potential to facilitate their effective clearance from tissues. SASP-induced antitumoural immune response is currently being studied through the specific recruitment of natural killer cells, cytotoxic T cells and M1 macrophages[37]. Furthermore, directed cellular approaches such as chimeric antigen receptor T (CAR T) cells might provide powerful strategies for increasing immunosurveillance. Indeed, it was demonstrated that senolytic CAR T cell therapy in mice, targeting the urokinase-type

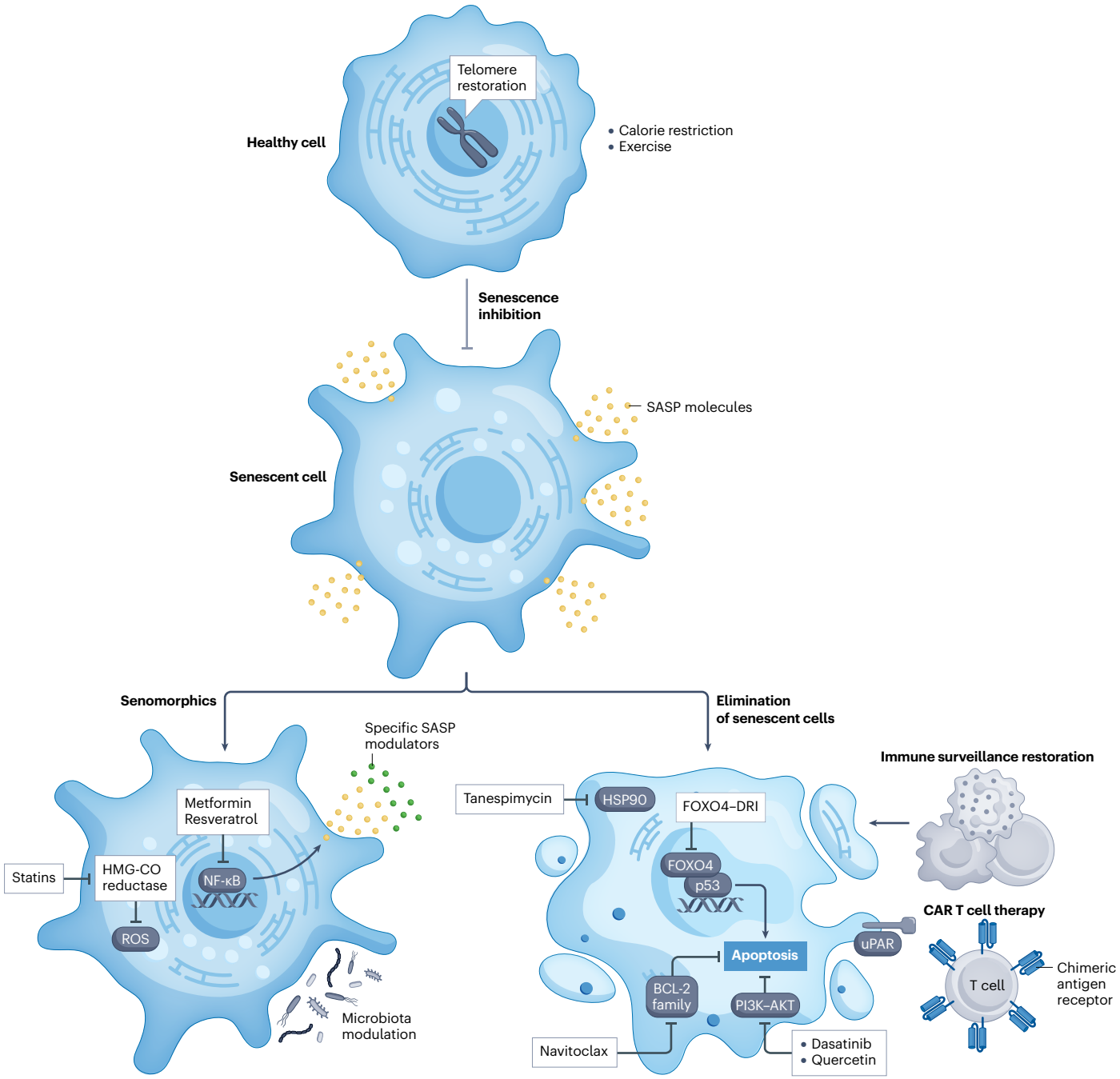

Fig. 2 | Therapeutic strategies targeting cell senescence in ageing and liver diseases. Senotherapies encompass various approaches to target cellular senescence, including the inhibition of senescence, modulation of the senescence-associated secretory phenotype (SASP) using senomorphics, and the specific elimination of senescent cells. Senolytics are utilized to clear senescent cells selectively. Dasatinib and quercetin promote senescence apoptosis by inhibiting the PI3K–AKT pathway. Navitoclax inhibits anti-apoptotic members of the BCL-2 family, inducing cell death through mitochondrial-mediated mechanisms. The senolytic peptide FOXO4–DRI disrupts the binding of FOXO4 to p53, releasing p53 to the cytoplasm and activating apoptosis. Inhibitors of HSP90, such as tanespimycin, have also been suggested to induce selective apoptosis of senescent cells. Other strategies to eliminate senescent cells involve immune-mediated clearance using chimeric antigen receptor T (CAR T) cells and restoring immune surveillance. Modulators of the SASP, such as metformin and resveratrol, inhibit NF-κB activity which reduces the pro-inflammatory ability of senescent cells. Statins can diminish the production of reactive oxygen species (ROS) by senescent cells. Modulation of the microbiota can reduce the levels of microbial compounds that stimulate the production of the SASP in senescent cells. Developing strategies aim to modulate specific SASP compounds to avoid off-target effects by suppressing cytokine secretion. Additionally, lifestyle interventions, such as calorie restriction, exercise and telomere restoration, have been shown to prevent senescence. FOXO4, forkhead box protein O4; DRI, D-retroinverso.

plasminogen activator receptor, attenuated senescence-associated diseases, including liver fibrosis[150].

Senomorphics

Instead of targeting senescent cells directly, which provides partial protection against tumour growth and is essential to retain liver function after hepatic injury, another therapeutic approach consists of interfering with the SASP components, which are believed to be detrimental, using senomorphics. For example, the inhibition of CCL2, which, as explained earlier, is key to recruiting $CCR2^+$ immature myeloid cells and maintaining their undifferentiated state[53], or the inhibition of IL-33 secreted by HSCs, which promotes HCC development[94], could be interesting targets to block senescence-induced tumour growth and avoid immune surveillance escape. Inhibiting TGFβ signalling in injured livers would prevent the spread of senescence from damaged hepatocytes to healthy and viable cells, facilitating the regeneration of injuries[108]. Such a treatment would be particularly beneficial in patients with CLD in whom liver regeneration is hindered, and would aid in the resolution of fibrosis.

Despite their advantageous applications, senomorphics exhibit a notorious limitation. In contrast to the intermittent administration of senolytics, which proves effective, the beneficial effects of senomorphics might necessitate regular and prolonged usage, which could potentially give rise to unforeseen adverse effects and induce off-target effects by suppressing cytokine secretion[151].

Other treatments to modulate liver senescence

Drug repurposing or repositioning has also emerged as a promising approach for developing senotherapeutics. This strategy offers a cost-effective and expedited pathway for identifying novel treatments and expanding the therapeutic options available for senescence-related disorders.

For instance, an in vitro study employing metformin, an antidiabetic medication with reported anti-ageing and antineoplastic effects, demonstrated its capacity to suppress the expression of various pro-inflammatory SASP molecules, leaving antitumoural factors unaffected. In addition, treatment of senescent cells with metformin disrupted support for cancerous cell growth caused by factors secreted by senescent cells through blockade of the NF-κB transcription factor[152]. The mechanism of action of metformin on senescence is through the activation of protein kinase AMP-activated catalytic subunit-α (AMPK), which inhibits the mammalian target of rapamycin complex 1 and induces peroxisome proliferator-activated receptor-γ coactivator 1-α, together with reduction in ROS production, and inhibition of pro-inflammatory cytokine signalling[153]. Interestingly, restoration of the senescent LSEC phenotype has also been observed with metformin treatment. In a preclinical study in young and aged mice, metformin ameliorated the typical changes in LSECs caused by age, including increased cell fenestrations, through upregulation in AMPK1 signalling, endothelial nitric oxide synthase (eNOS) and myosin light chain kinase phosphorylation[154].

Furthermore, statins, which are hydroxymethylglutaryl-CoA reductase inhibitors and are widely employed for reducing cholesterol levels in patients diagnosed with hypercholesterolaemia, were reported to inhibit oxidative stress derived by endothelial senescence in diabetic mice and in human umbilical vein endothelial cells[155]. In the liver, statins enhance endothelial function by increasing the activity of eNOS and nitric oxide availability. In cirrhotic livers of rats, this effect was achieved by activating KLF2, leading to reduced HSC activation and decreased portal hypertension[156,157]. Regarding hepatocytes, the transplantation of healthy, non-senescent hepatocytes into the liver of rats prone to HCC proved to be an effective treatment. Healthy hepatocytes facilitated the clearance of tumour-promoting senescent hepatocytes, thereby reducing the rate of HCC development[158].

In addition to specific treatments or therapeutic approaches using pharmaceutical compounds, other therapies to counteract liver senescence focusing on the diet, the gut microbiota or lifestyle interventions are also being studied. For instance, exercise and calorie restriction in a preclinical model of HFD-induced MASLD led to amelioration in liver senescence and steatosis as a result of an increase in liver lipophagy[159]. Similar effects were obtained with moderate exercise in rats, with the researchers attributing these effects to an increase in NAD^+ levels in the liver[160]. Although the specific effects of exercise on cellular senescence are not fully known, it is thought to prevent the accumulation and promote the clearance of senescent cells — different clinical and preclinical studies have found that exercise is linked to decreased markers of cell senescence[161].

The use of natural bioactive compounds, such as resveratrol, as a therapeutic approach to modulate senescence has also gained marked attention owing to their antioxidant and anti-inflammatory effects. Resveratrol is a polyphenol found in grapes, berries, peanuts and red wine, with reported anti-ageing effects[162]. Specifically, resveratrol treatment in vitro restored LSEC capillarization induced by liver senescence. These effects were induced by the upregulation of SIRT1, which modulates cell senescence[163]. Also, other clinical and preclinical studies have found that resveratrol targets other parameters in the liver that are usually altered due to senescence, such as oxidative stress, mitochondrial dysfunction and lipid accumulation[164–166]. Curcumol, a sesquiterpenoid found in turmeric and plants from the same family, was found to be capable of protecting against hepatocyte senescence in a preclinical model of MASLD, which helped in the resolution of liver steatosis and injury[167]. Further, a study showed that curcumol, both in vivo and in vitro, regulates TERT and telomeric repeat binding factor 1 gene and protein expression levels, among other senescence-related factors, which protects telomeres. This mechanism is believed to be induced by the protective effects of curcumol on iron overload, which is upregulated in MASLD and causes ROS accumulation, which induces cell senescence[167].

Treatments targeting the gut microbiota might also be effective in treating liver senescence and age-related diseases. For instance, as mentioned above in the section 'Hepatic stellate cells', Yoshimoto and colleagues, in a preclinical model of obesity and HCC, found that inhibiting deoxycholic acid, a compound derived from a specific genus of gut bacteria, leads to a decrease in HCC emergence. This effect was attributed to the ability of this gut microbiota-derived compound to induce SASP expression in HSCs, thereby promoting inflammation and tumorigenesis[111]. These findings highlight the role of gut microbiota modulation as a potential therapeutic avenue for liver diseases and other age-related disorders.

Telomere restoration could also be an interesting therapy for CLD-induced liver senescence. In a study in mice, restoring telomere function improved liver cell regeneration in cirrhotic livers and decreased TGFβ signalling and HSC activation[50]. Similarly, the improvement in telomerase activity prevented HFD-induced telomere shortening, which ameliorated MASLD[168], and the exogenous delivery of the human *TERT* gene into rats before liver transplantation alleviated ischaemia–reperfusion injury, especially in aged livers[169].

Senescence induction to treat liver diseases and HCC

Although tumoural cells have escaped senescence activation, studies have shown that they can still be induced, posing an attractive therapeutic approach for HCC treatment[170]. Currently, a two-step approach has been proposed, which involves senescence induction in malignant cells, followed by the administration of a senolytic agent. For instance, in an in vivo and in vitro study, the arrest of DNA replication via cell division cycle 7 inhibition, followed by treatment with the repurposed anti-depressant drug, sertraline, which effectively suppresses mTOR signalling, led to apoptosis induction and subsequent inhibition of tumour growth[171].

Inducing controlled senescence in the tumoural site has also been shown as a promising therapeutic option, activating key pro-inflammatory cytokines and surface markers that activate natural killer cells that clear tumoural cells[172]. Regarding senescence induction in HCC, the in vivo therapeutic activation of p53 was capable of stunting tumour growth, firstly by inducing cell cycle arrest and secondly by promoting the secretion of inflammatory cytokines that signal the immune response to clear tumoural-senescent cells[27]. TGFβ, a robust inhibitor of hepatocyte cell cycle progression, has also demonstrated therapeutic potential in managing HCC, triggering cellular senescence in tumour cells and exerting inhibitory effects on tumour growth in murine models[173].

Differently, induction of IL-22, which has been found to induce senescence of HSCs in mice[98], might be an effective antifibrotic treatment[174–176]. Also, reprogramming the SASP through activating or inhibiting particular cytokines is another proposed treatment that could enhance the recruitment of antitumoural immune cells and inhibit the expression of factors that stimulate tumour growth[177].

Collectively, the major challenge for potentiating senescence as a safeguard in cancer prevention is to develop senescence inducers that specifically target organs or cell types of interest, which requires further screening of potential chemical or biological candidates in various preneoplastic liver diseases.

Conclusions

The growing recognition of the substantial impact of cellular senescence on the pathogenesis and progression of liver diseases has led to extensive efforts to unravel the intricate mechanisms underlying this phenomenon. As described above, current research is underway to elucidate the factors and signalling pathways that induce senescence in both parenchymal and non-parenchymal liver cells, aiming to pinpoint specific triggers that promote the onset of this cellular state. By understanding the precise mechanisms that initiate and maintain cellular senescence, the hepatology community might be able to identify novel therapeutic targets and develop strategies to intervene at different stages of the senescence process for a variety of liver diseases, including steatosis, fibrosis and cancer. Moreover, to advance our comprehension of senescence and its role in health and disease and to assess the efficacy of senotherapeutic interventions, establishing precise senescence detection methodologies is of vital importance.

Another promising avenue of research focuses on developing targeted therapies that eliminate senescent cells in the liver. These therapies, referred to as senolytic treatments, aim to eliminate the senescent cells contributing to disease progression selectively. By removing these cells, it is hypothesized that liver function could be improved, fibrosis could be attenuated, and the overall course of liver diseases could be altered. Various approaches are being explored, including using small molecules, known drugs and gene therapies that specifically target and eliminate senescent cells without harming healthy cells. This approach could also be achieved using novel nanoformulations such as quantum dots or nanoparticles that could be administered systemically but would specifically target the desired cell type[178]. In addition to targeting senescent cells directly, novel senomorphic strategies to prevent or modulate the SASP are being developed. By targeting SASP components, it might be possible to mitigate the chronic inflammation and fibrotic processes associated with liver diseases, potentially leading to improved patient outcomes.

To conclude, the ongoing research in the field of cellular senescence in liver diseases holds immense potential for the development of innovative therapeutic approaches. By gaining a deeper understanding of the molecular mechanisms driving senescence and its consequences, we are paving the way for the discovery of novel drug targets and therapeutic interventions. The ultimate goal is to develop effective and safe treatments to manage and treat liver diseases and enhance the quality of life of individuals affected by these conditions.

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Author contributions

D.S.-R. and A.G.-R. researched data for the article. J.G.-S. conceptualized and coordinated the work. All authors contributed substantially to discussion of the content. All authors wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

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